REVIEW ARTICLE

Brown and Beige Fat: Therapeutic Potential in Obesity

Anna Meiliana[1,2,*], Andi Wijaya[2,3]

[1]Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia
[2]Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia
[3]Postgraduate Program in Clinical Biochemistry, Hasanuddin University, Jl. Perintis Kemerdekaan Km.10, Makassar, Indonesia
*Corresponding author. E-mail: anna.meiliana@prodia.co.id

Abstract

BACKGROUND: The epidemic of obesity and type 2 diabetes presents a serious challenge to scientific and biomedical communities worldwide. There has been an upsurge of interest in the adipocyte coincident with the onset of the obesity epidemic and the realization that adipose tissue plays a major role in the regulation of metabolic function.

CONTENT: Adipose tissue, best known for its role in fat storage, can also suppress weight gain and metabolic disease through the action of specialized, heat-producing adipocytes. Brown adipocytes are located in dedicated depots and express constitutively high levels of thermogenic genes, whereas inducible 'brown-like' adipocytes, also known as beige cells, develop in white fat in response to various activators. The activities of brown and beige fat cells reduce metabolic disease, including obesity, in mice and correlate with leanness in humans. Many genes and pathways that regulate brown and beige adipocyte biology have now been identified, providing a variety of promising therapeutic targets for metabolic disease.

SUMMARY: The complexity of adipose tissue presents numerous challenges but also several opportunities for therapeutic intervention. There is persuasive evidence from animal models that enhancement of the function of brown adipocytes, beige adipocytes or both in humans could be very effective for treating type 2 diabetes and obesity. Moreover, there are now an extensive variety of factors and pathways that could potentially be targeted for therapeutic effects. In particular, the discoveries of circulating factors, such as irisin, fibroblast growth factor (FGF)21 and natriuretic peptides, that enhance brown and beige fat function in mice have garnered tremendous interest. Certainly, the next

Abstrak

LATAR BELAKANG: Epidemi obesitas dan diabetes tipe 2 menghadirkan tantangan yang serius bagi komunitas ilmiah dan biomedik di seluruh dunia. Terjadi peningkatan minat terhadap adiposit, bersamaan dengan onset epidemi obesitas dan kesadaran bahwa jaringan adiposa memainkan peran utama pada pengaturan fungsi metabolisme.

ISI: Jaringan adiposa, terkenal karena fungsinya sebagai penyimpanan adiposa, juga dapat menekan kenaikan berat badan dan penyakit metabolisme melalui adiposit khusus yang menghasilkan panas. Adiposit coklat terletak pada cadangan tertentu dan secara terus menerus mengekspresikan gen termogenik dalam kadar tinggi, sementara itu adiposit "*brown-like*", yang dikenal sebagai sel *beige*, berkembang di dalam jaringan adiposa putih sebagai respon dari berbagai aktivator. Aktivitas sel adiposa coklat dan *beige* mengurangi penyakit metabolisme, termasuk obesitas, pada tikus dan berhubungan dengan kerampingan pada manusia. Beberapa gen dan jalur yang mengatur adiposit coklat dan *beige* telah teridentifikasi, dan menjadi target terapi yang menjanjikan bagi penyakit metabolisme.

RINGKASAN: Kompleksitas jaringan adiposa menghadirkan berbagai tantangan dan juga kesempatan bagi terapi intervensi. Beberapa bukti persuasif pada hewan coba menunjukkan bahwa peningkatan fungsi adiposit coklat, *beige*, atau keduanya pada manusia sangat efektif untuk mengobati diabetes tipe 2 dan obesitas. Lebih lagi, ada berbagai faktor dan jalur ekstensif yang berpotensi sebagai target terapi. Secara khusus, penemuan faktor sirkulasi seperti irisin, *fibroblast growth factor* (FGF)21, dan *natriuretic peptide*, yang mampu meningkatkan fungsi adiposa coklat dan *beige*, telah menarik banyak perhatian.

decade will see massive efforts to use beige and brown fat to ameliorate human metabolic disease.

KEYWORDS: obesity, white adipose tissue, brown adipose tissue, beige adipose tissue, adipose organ, thermogenesis, energy expenditure

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Tentu saja, dekade berikut akan menunjukkan usaha besar-besaran penggunaan adiposa coklat dan *beige* untuk mengatasi penyakit metabolisme.

KATA KUNCI: obesitas, jaringan adiposa coklat, jaringan adiposa *beige*, organ adiposa, thermogenesis, pengeluaran energi

Introduction

The epidemic of obesity and diabetes has greatly increased the interest in brown fat. Adipocytes can be broadly divided into white and brown fat cells. Whereas white fat cells are specialized to store chemical energy, brown adipocytes produce heat, counteracting hypothermia, obesity, and diabetes. Brown fat generates heat via the mitochondrial uncoupling protein 1 (UCP1), defending against hypothermia and obesity. Recent data suggest that there are two distinct types of brown fat: classical brown fat derived from a myf-5 cellular lineage and UCP1-positive cells that emerge in white fat from a non-myf-5 lineage.(1)

Metabolically active brown adipose tissue (BAT) depots have been detected in adult humans by positron emission tomography/ computed tomography with the glucose analog F18-fluorodeoxyglucose (FDG-PET/ CT). Human BAT is located mainly to the cervical, supraclavicular, and paravertebral areas and can be activated by cold.(2-7) The ‘‘brown-like’’ cells within white adipose depots are not derived from the myf-5 lineage and have been called ‘‘beige’’ or ‘‘brite’’ cells.(8-10) Interestingly, it has been reported that distinct genetic loci control the amounts of UCP1-positive cells in the white and classical brown fat depots (11-15), strongly suggesting that these two types of thermogenic cells are regulated differently. The therapeutic potential of both kinds of brown fat cells is clear (10,16), as genetic manipulations in mice that create more brown or beige fat have strong antiobesity and antidiabetic actions.

It was recently proposed that supraclavicular fat of adult humans consists of beige rather than classical brown fat.(1,17) However, in order to exclude the possibility of the presence of classical brown fat in adult humans, the newly established markers must be compared with well-established markers in a larger sample set. This is important, as it is possible that the regulation and function of brown and beige adipose tissue differ, and the mode of recruiting the tissue for counteracting the development of obesity might depend on the nature of the tissue.(18) The hope is that molecules that activate brown fat or induce the conversion of white fat to brown-like fat could help treat metabolic diseases such as obesity and diabetes.

Adaptive Thermogenesis (AT) and Weight Loss

Cellular bioenergetics are considered as a suitable target for antiobesity therapy. The capacity of energy production, heat release, and metabolic adaptations to either starvation, diet, cold, stress, and inflammation differ between subjects. Although the metabolic basis of these adaptations as well as the intraindividual and interindividual variances between them still remain to be characterized, modulation of AT to affect energy balance is challenging; its main regulators, that is, either sympathetic nervous system (SNS) activity, 3,5,3’-tri-iodothyronine (T3), and/or leptin may provide a suitable basis for pharmacotherapies.(19)

AT has been defined as the change in energy expenditure following acute and/or long-term overfeeding and underfeeding. In some studies, it has also been investigated by using catecholamines, caffeine, ephedrine and adrenergic blockers to induce changes in thermogenesis. As previously described (20), these stimuli were found to induce statistically significant changes in thermogenesis. (21) AT may represent a defense mechanism that is set to protect energy stores from accelerated growth or depletion. The first explanation that has been proposed to explain thermogenic variations in animals is related to the activity of BAT. As described by Rothwell and Stock (22), BAT activity has sufficient impact on energy metabolism to explain individual variations in the proneness to obesity. Beyond the demonstration of validity of the greater-than-predicted change in energy expenditure as a measure of adaptive thermogenesis (23), Rosenbaum *et al*. (24) have also contributed to identify some metabolic correlates of this variable. Indeed, a decrease in thermogenesis in weigh-reduced obese individuals was found to be associated with a decrease in plasma T3 and leptin, as well as sympathetic nervous system activity.(24)

Although weight reduction is a difficult task, the maintenance of lost weight seems to require the deployment of even more efforts.(25) Indeed, the relapse of more than 80% of individuals to pre-weight-loss levels of body fatness after otherwise successful weight loss is likely due to the coordinated actions of metabolic, neuroendocrine, autonomic and behavioral changes that oppose the maintenance of a reduced body weight.(26) The occurrence of an apparent resistance to lose fat (plateau) is often interpreted as being the result of a lack of dietary and/or physical activity guidelines compliance. However, the adaptive reduction in thermogenesis can be sufficiently pronounced in some cases to counteract further weight loss, even in the compliant patients.(20,27,28)

As these thermogenic changes would seem to persist over time, they likely contribute to body weight regain following body weight loss. It thus seems important to further investigate adaptive thermogenesis in humans, be it for the development of relevant biomarkers or to improve diagnosis about individual determinants of the predisposition to obesity.(21) The existence of beige fat cells may represent an evolutionarily conserved cellular mechanism to provide flexibility in adaptive thermogenesis. It is likely that these beige adipocytes, rather than the classical brown fat cells, remain present in the adult state of larger mammals in which hypothermia is a less frequent threat than in rodents.(1) The therapeutic potential of activating brown-fat-mediated thermogenesis in human has yet to be fulfilled. Trials of drugs that increase the b-adrenergic activation of BAT have not been successful in humans due to either lack of efficacy or to intolerable side effects resulting from activation of β-adrenergic receptors in other tissues. Clearly, there is a need to develop more specific means to activate brown fat in humans in more specific ways.(29,30)

BAT

The most important single idea in the field of metabolic disease is the concept of energy balance. This means that, with the rare exception of mal-absorption of nutrients, an animal cannot gain or lose weight unless there is an imbalance between food intake and energy expenditure. When energy intake chronically exceeds energy expenditure, weight gain and obesity result. This excess weight is stored in adipose tissue, which consists of fat cells, or adipocytes, which have an incredible capacity for storing surplus energy in the form of lipid. This tissue is not just a passive storage depot, but also an endocrine organ, secreting molecules like leptin that can regulate appetite and whole-body metabolism. In addition to these well-described energy-storing fat cells, adipocytes also exist that are highly effective at transforming chemical energy into heat. Brown adipocytes, which get their name from their high number of iron-containing mitochondria, are specialized to dissipate energy in the form of heat, a process called non-shivering thermogenesis. Classical BAT is typically located in the interscapular region and is most easily detected in infants and small mammals. It is referred to as ‘‘classical’’ in distinction from the inducible or beige adipocyte, which has unique molecular and developmental characteristics.(31) BAT specializes in burning fat and is responsible for adaptive, nonshivering thermogenesis in mammals.(32)

UCP1 is responsible for non-shivering thermogenesis in BAT. Upon activation by long-chain fatty acids (LCFAs), UCP1 increases the conductance of the inner mitochondrial membrane (IMM) to make BAT mitochondria generate heat rather than adenine triphosphate (ATP).(33) BAT burns fatty acids for heat production to defend the body against cold (32,34) and has recently been shown to be present in humans. (3,4,35) Triglyceride-rich lipoproteins (TRLs) transport lipids in the bloodstream, where the fatty acid moieties are liberated by the action of lipoprotein lipase (LPL).(36) Increased BAT activity induced by short-term cold exposure controls TRL metabolism in mice. Cold exposure drastically accelerated plasma clearance of triglycerides as a result of increased uptake into BAT, a process crucially dependent on local LPL activity and trans-membrane receptor CD36. In pathophysiological settings, cold exposure corrected hyperlipidemia and improved deleterious effects of insulin resistance.(37)

BAT is a highly energetic organ that not only utilizes its unique expression of UCP1 for uncoupling of respiration (*i.e.*, cold or diet-induced thermogenesis), but is also a mitochondrially rich tissue that uses glucose and fatty acids as a fuel.(3,37-39) Adult humans have substantial depots of metabolically active BAT (3-5,7), suggesting that BAT may play a fundamental role in the maintenance of a leaner and more metabolically healthy phenotype.

Cold exposure is the most powerful and physiological stimulus for BAT activation, both in small rodents and in humans.(7,40-44) It is known that the stimulatory effects of cold on BAT are mediated through the activation of the sympathetic nervous system, initiated by peripheral stimulation of transient receptor potential (TRP) channels in sensory neurons.(41,45) This pathway is also activated by some food ingredients, such as capsaicin and capsinoids, nonpungent capsaicin analogs.(46,47) Stimulation of TRP channels by capsinoids is effective for enhancement of BAT thermogenesis and upregulation of UCP1, a key molecule

of BAT thermogenesis, in mice.(47) It is therefore expected that the reactivation and/or recruitment of BAT may protect against the onset of obesity and related metabolic disorders in humans.(48)

Beige Adipose Tissue

The last few years have seen a paradigm shift with regard to the understanding of the origin of and relationship between adipose tissues. A series of investigations have demonstrated that brown and white adipocytes are not sister cells, but rather, brown adipocytes are closely related to myocytes, and both originate from a common "adipomyocyte" precursor.(10,49,50) Furthermore, even among classical white adipocytes, it would seem that two types exist: the "genuine" white adipocytes and also "beige" adipocytes, a type of adipocyte that possesses the ability to express the UCP1, which until recently was believed to be a unique marker for brown adipocytes, but does not otherwise possess the full molecular characteristics of brown adipocytes. (9,10,12)

Recent studies found that each adipose depot examined was characterized by a unique marker gene expression pattern. However, despite this, we conclude that it is possible to divide the depots into three main types, the classical BAT depots, the beige adipose tissue depots, and the ("genuine") white adipose tissue (WAT) depots, and to associate particular gene expressions patterns with these depots.(51) It is now clear that there are two distinct types of brown adipose cells. One is the classical brown fat that arises from a myogenic factor 5 (myf5), muscle-like cell lineage.(10,52) This is exemplified by the interscapular BAT depots and the perirenal BAT depot, a depot that contains a mixture of classical brown and white adipocytes. (10,53) In addition, UCP1-positive, brown fat-like cells can emerge in most WAT depots upon prolonged cold exposure or β-adrenergic receptor activation.(54-56) These brown fat cells, which are not derived from a myf5-positive lineage, are designated beige or brown-in-white (beige) cells.(9,57)

Compared with WAT, BAT is more extensively vascularized, and VEGF-α–dependent angiogenesis has been shown to be important for the thermogenic response to prolonged cold adaptation. (59) However, the role of BAT vascularity in maintaining systemic metabolic homeostasis under conditions of metabolic stress is unknown. Here, we show that obesity causes capillary rarefaction and hypoxia in BAT that is much more robust than in WAT. This condition leads to BAT "whitening" that is associated with diminished β-adrenergic signaling, the accumulation of large lipid droplets, and mitochondrial dysfunction and loss. These changes in the BAT microenvironment impair thermogenic responses and contribute to dysfunctional glucose metabolism.(60) Browning of rodent WAT can be brought about by hormones and cytokines, such as Irisin (61) and fibroblast growth factor (FGF) 21 (62), as well as by transcriptional modulation through PR domain containing (Prdm)16 (57), Forkhead box protein C2 (FoxC2) (63), receptor-interacting protein 140 (RIP140) (64), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (65), transcriptional mediators/intermediary factor 2 (TIF2) (66), retinoblastoma protein (pRb) and p107 (67). However, there is an unmet need for strategies that would translate these mechanisms into the clinic. (68)

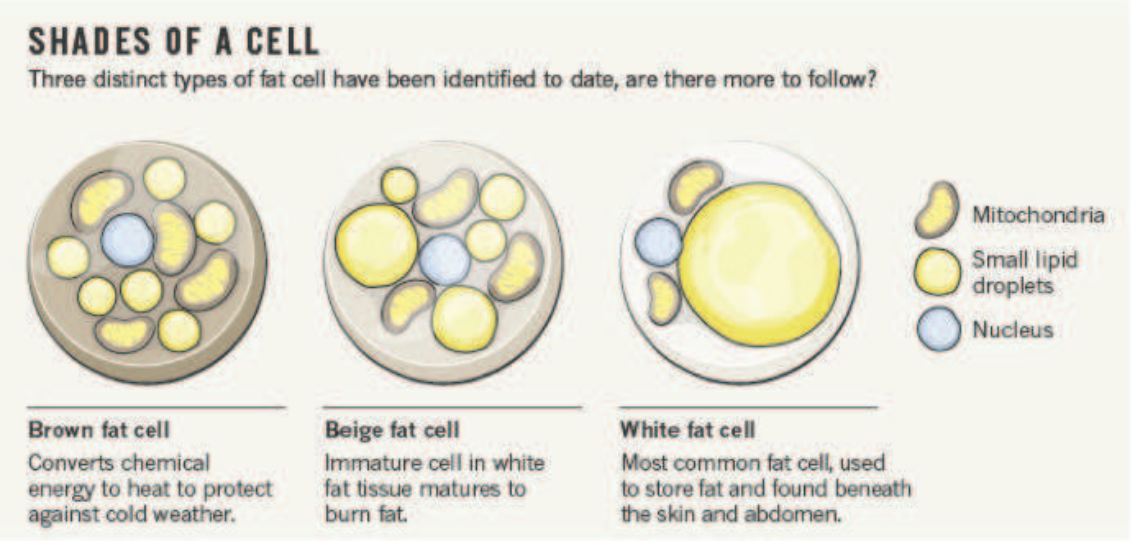

Figure 1. Shades of a cell.(58) (Adapted with permission from Nature Publishing Group).

Adipose Organ Plasticity

White adipose cells in mammals have the ability to store and release energy in the form of lipids. This function is essential as it allows intervals of fasting between meals and prolonged, week-long, fasting intervals.(69,70) The large spherical shape of white adipocytes fits perfectly with their storage function because this geometric shape allows for maximum storage in minimal space. More than 90% of the volume of white adipocytes is occupied by a cytoplasmic unilocular lipid droplet consisting of triglycerides. White adipocytes are also able to secrete hormones (71) and several cytokines that influence instinctual behaviour and metabolic pathways (70).

Brown adipocytes are thermogenic cells that are highly regulated by the sympathetic nervous system to maintain body temperature when mammals are exposed to temperatures below thermoneutrality (approximately 28-30°C).(72) To produce heat, brown adipocytes are activated by sympathetic nerves acting on beta3-adrenoceptors (beta3AR) to burn fatty acids in their unique spherical mitochondria, where the UCP1 protein in the inner membrane uncouples oxidative phosphorylation, resulting in energy dissipation in the form of heat.(32,73) Brown adipocytes store triglycerides in multiple small cytoplasmic lipid droplets so that they can rapidly burn large amounts of fatty acids.(74)

Brown and white adipocytes appear to have different morphology and opposing functions, but detailed anatomic studies have shown that both types are contained together in several locations, forming a multi-depot adipose organ (75,76). Discrete depots are located in the subcutaneous space (subcutaneous depots) or in close vicinity to organs located in the trunk (visceral depots).(77) This finding has opened new perspectives in the physiological relationship between BAT and WAT, including the possibility of their reciprocal transformation (transdifferentiation) (56,66,78). Harnessing the mechanism of WAT to BAT transdifferentiation could be useful to develop treatments for obesity and type 2 diabetes, because the absence of BAT or its adrenergic receptors results in obesity (79,80) and transgenic mice overexpressing UCP1 in WAT are obesity resistant. (81,82)

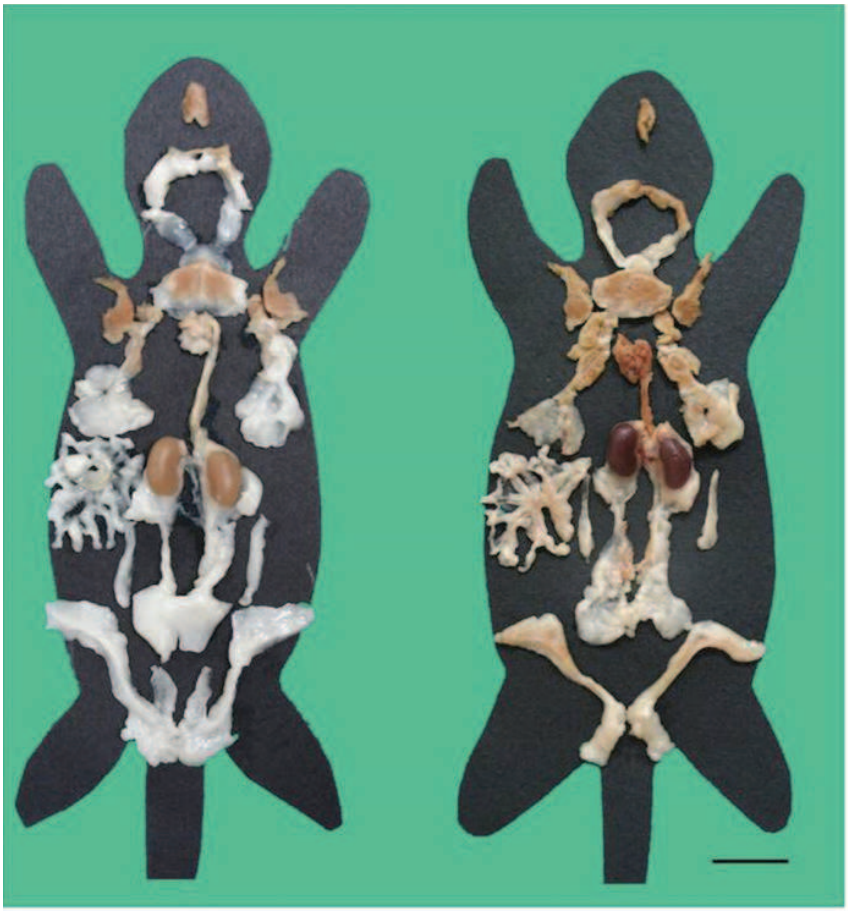

Figure 2. Multidepot adipose organ of adult C5 & BL/6J female mice kept at 28°C (left) or 6°C (right) for 10 days.(82) (Adapted with permission from American Society for Biochemistry and Molecular Biology).

Humans and mice harbour brown-like adipocytes in their predominantly white fat depots. (54,83) These beige (brown-in-white) adipocytes are also known as beige, inducible brown or brown-like adipocytes. (1,84,85) The abundance of this highly dynamic cell population is strain- and location-dependent (12,86) and it increases (beigening) in the cold whereas it decreases (whitening) in a warm environment (83,87). The inguinal (posterior subcutaneous) depot constitutes the best studied and, owing to its size, the most important fat tissue capable of recruiting beige adipocytes.(51) Each depot is associated with specific nerve-vascular peduncles as are other organs in the body. Some researchers prefer to call these depots 'mini-organs' because of their autonomous anatomy and characteristics (88). Cinti *et al*. prefer the term 'adipose organ' to describe the general functional and plastic properties that are shared by most of its depots.(74)

Intermediate forms of adipocytes between white and brown adipocytes are present in all depots of the adipose organ.(82,89) These intermediate adipocytes are multilocular but do not express the UCP1 protein, as indicated by immunohistochemistry. They also have mitochondria with intermediate features between those found in white and brown adipocytes. These cells could have a predominant lipid droplet and have been called paucilocular adipocytes.(56) Paucilocular adipocytes have a weak thermogenic capacity, in line with the idea that they are intermediate forms between white and brown adipocytes. These intermediate adipocytes are usually located in areas between WAT and BAT.(82)

The reason why white and brown adipocytes, with different morphology and physiology, are contained together in the same organ is unclear, but it should be noted that they both share important features, such as the ability to store and release lipids and the expression of beta3AR. (90,91) Cinti *et al.* proposed a trans-differentiation theory: in specific physiologic conditions (chronic cold exposure), white adipocytes transform into brown adipocytes to supply the thermogenic needs, and conversely, brown adipocytes transform into white adipocytes when the energy balance is positive and the adipose organ requires increased storage capacity.(92) This plasticity is not limited to these conditions because during pregnancy, lactation or post-lactation states in females, white adipocytes seem to have the ability to convert into milk-secreting epithelial cells

Thus, adult humans seem to have expandable metabolically active brown adipocytes. In addition, it has been proven that animals with abundant natural (82) or induced (81,93) BAT are resistant to obesity and that the administration of drugs that expand BAT is sufficient to curb obesity and related disorders in rats. (55,94) Conversely, mice without functional brown adipocytes are prone to obesity and type 2 diabetes. (79,80) The white / brown plasticity of adipose tissue might have considerable medical implications, since the brown-like phenotype seems to correlate with a reduced propensity for developing obesity and diabetes in mice. (79-81,95,96)

Therapeutical Potential of Brown and Beige Fat

The biomedical interest in brown and beige adipocytes has centered on the capacity of these cell types to counteract metabolic disease, including obesity and type 2 diabetes. Indeed, increased activities of brown and beige adipocytes have been linked to obesity resistance in many mouse models. (57,63,81) The key question now is whether reduced thermogenic activity in fat cells is a cause or a consequence of weight gain in humans. Regardless of its natural role, increasing the activity of brown fat, beige fat or both through drugs or other methods holds tremendous promise for the treatment of metabolic disease.(97)

Most brown fat cells originate from precursor cells in the embryonic mesoderm that also give rise to skeletal muscle cells and a subpopulation of white adipocytes. (10,52,98) These precursors transiently express Myf5 and paired box (Pax),(82) two genes that were previously thought to selectively mark skeletal myogenic cells in the mesoderm. (10,52) Consistent with a developmental relationship between brown fat and muscle, brown fat precursor cells express a muscle-like gene signature, (50) and brown fat and muscle have related mitochondrial proteomes. (99) The embryonic origin and cell hierarchy of beige adipocytes is less clear. Beige and brown adipocytes probably come from distinct cell lineages, given that beige cells, at least in the subcutaneous depot, do not have a history of Myf5 expression.(10,98)

Over a decade ago, Himms-Hagen *et al*. (77) found that most beige adipocytes arise from pre-existing (non-dividing) cells that they presumed were mature adipocytes. Since then, Cinti and others have provided substantial evidence in support of the idea that large unilocular white adipocytes transform into beige adipocytes in response to cold or β3-adrenergic agonists. (82) Wang *et al.* indicates that most, if not all, beige adipocytes (at least in this subcutaneous depot) arise from a precursor population rather than from pre-existing adipocytes.

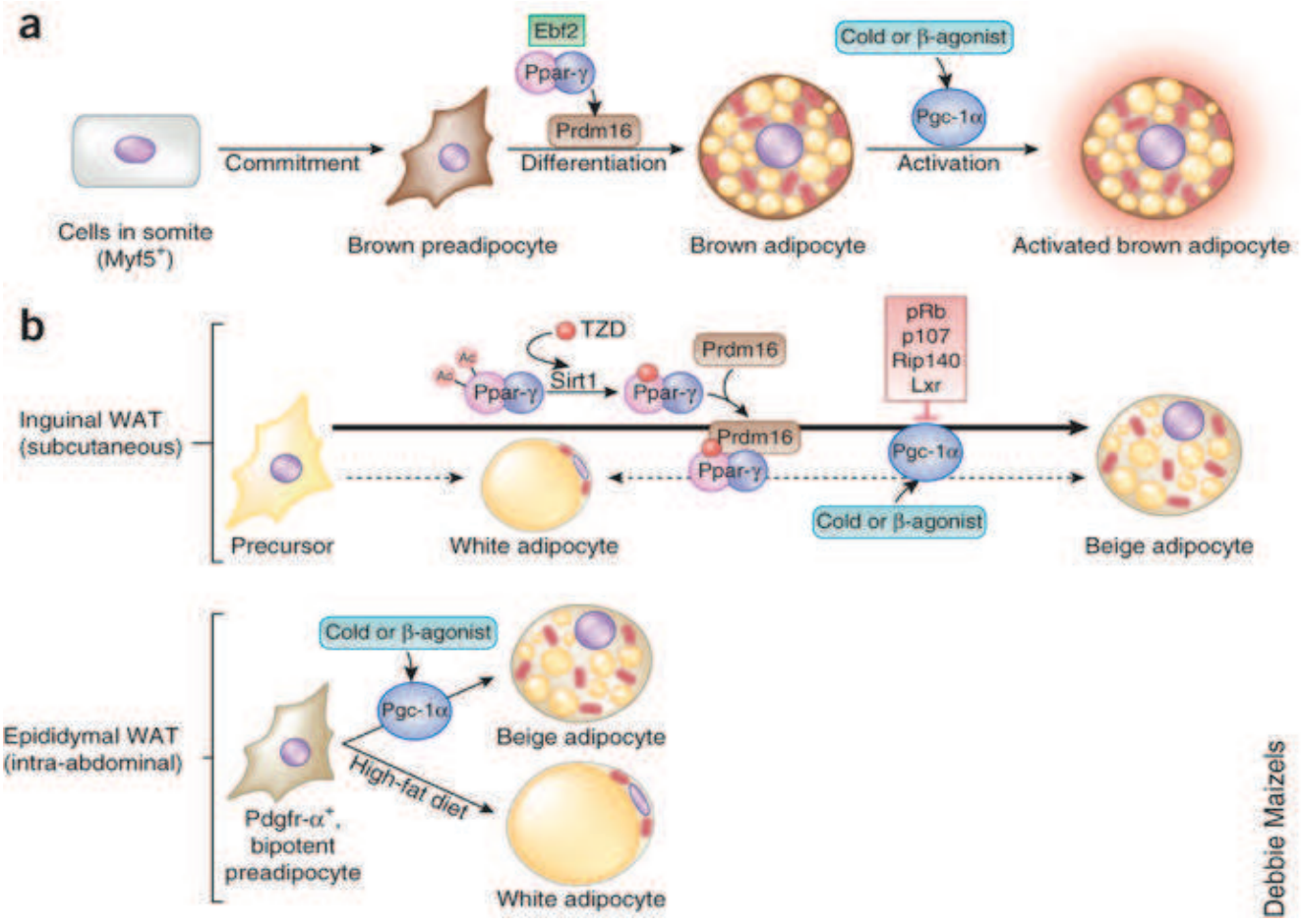

Figure 3. Transcriptional regulation of brown and beige adipocyte development.(97) (Adapted with permission from Nature Publishing Group).

The thermogenic profile of beige adipocytes is reversible. Beige adipocytes acquired in WAT during cold exposure lose UCP1 expression and are retained after mice are moved back to warmer conditions. When these mice are re-exposed to cold, the same cells again induce UCP1 expression.(101) Interestingly, the cells marked by previous UCP1 expression were not the only source of beige adipocytes during the second round of cold exposure. This suggests that beige adipocytes are retained and may function similarly to white fat cells for a certain period of time in animals that were previously cold. These beige adipocytes are presumably depleted through the normal mechanisms that control tissue turnover.(97)

Prdm16 is a large zinc finger–containing transcriptional factor that is highly expressed in mouse BAT relative to visceral WAT.(102) Prdm16 expression is also substantially enriched in human BAT relative to adjacent subcutaneous WAT. (5,103) Several factors have been shown to regulate brown and beige adipocyte differentiation by modulating Prdm16 expression or activity. Notable among these factors is bone morphogenetic protein 7 (BMP7), a signal that is essential for brown fat development, which increases the amounts of Prdm16 mRNA in brown and white fat precursor cells.(84,104,105) Additionally, thiazolidinediones (TZDs), which agonize peroxisome proliferator-activated receptors (PPAR)-γ, induce thermogenic gene expression in fat cells through effects on Prdm16.(48,106)

Mice with increased activity of brown fat, beige fat or both resist weight gain but also display improvements in systemic metabolism, including improved glucose tolerance and increased insulin sensitivity.(61,63,107,108) Along these lines, activated brown fat takes up and metabolizes large quantities of lipid from the bloodstream, (37) which has beneficial effects on metabolism. The increased proportion of beige to white adipocytes in WAT modulates systemic insulin action through nonthermogenic mechanisms, perhaps by altering the secretome of adipose tissue. Additionally, thermogenic fat cells, not yet classified as brown or beige, that surround blood vessels (perivascular adipose) have been suggested to protect against the development of atherosclerosis.(109) Thus, the potential therapeutic uses of brown and beige fat go beyond obesity and should be

considered for various metabolic disturbances, including type 2 diabetes, insulin resistance, atherosclerosis and lipid disorders.(97)

Pharmacological and Nutritional Agents Promoting Browning of WAT

Increasing energy expenditure is an attractive approach to fighting the worldwide epidemic in obesity and type 2 diabetes. Exercise is an important component of good health and represents the first line of therapy for humans with a variety of metabolic disorders: obesity, diabetes, and nonalcoholic hepatic steatosis. Recent data has shown that exercise, besides using calories to do physical work, also causes an increase in energy expenditure through augmentation in brown fat and the browning of white fat. (61,110) Indeed, these effects on brown fat could represent part of the longer-lasting benefits of exercise.

That brown fat, in all of its dimensions, can improve type 2 diabetes and metabolic health seems to be settled science, at least in experimental animals. These cells express UCP1 and have a high mitochondrial content, thereby dissipating chemical energy in the form of heat. In fact, the improvements seen in glucose tolerance observed with "browning" of white fat and the formation of "beige" or "brite" cells might be greater than expected solely from their effects on body weight and adiposity.(111) Several polypeptides, including FGF21, BMP7/8b, brain natriuretic peptide (BNP)/atrial natriuretic peptide (ANP), and orexin, all have interesting browning effects. (62,104,108,112,113)

Irisin was of interest because it is induced during exercise in rodents and is at least partially responsible for the browning response observed in white fat during chronic exercise.(61) The parent polypeptide, fibronectin type III domain containing 5 (FNDC5), is synthesized as a type 1 membrane protein and is then cleaved and shed into the circulation as a highly glycosylated polypeptide of roughly 12 kDa. Irisin appears to act preferentially on the browning of white fat deposits when elevated in the blood of obese mice via viral vectors. This correlates with improvements in glucose tolerance in obese mice. Regarding human irisin, it is clear that FNDC5 mRNA is increased in skeletal muscle in some exercise paradigms but not others. (61,114,115) Interestingly, two articles report that human patients with diabetes are deficient in irisin compared with normal counterparts.(116,117)

FGF21 is a recently discovered metabolic regulator. Exogenous FGF21 produces beneficial metabolic effects in animal models; however, the translation of these observations to humans has not been tested. Gaich *et al.* studied the effects of LY2405319, a variant of FGF21, in a randomized, placebo-controlled, double-blind proof-of-concept trial in patients with obesity and type 2 diabetes. The results indicate that FGF21 is bioactive in humans and suggest that FGF21-based therapies may be effective for the treatment of selected metabolic disorders.(118)

Atrial natriuretic peptide and brain-type natriuretic peptide are released by the heart in response to heart failure or pressure overload. These factors reduce blood volume, blood pressure and cardiac output by dilating blood vessels and promoting salt and fluid excretion from the kidneys. Atrial natriuretic peptide is also known to promote lipolysis in adipocytes. Notably, high circulating concentrations of natriuretic peptides have also been associated with weight loss in humans.(119,120) The effects of natriuretic peptides on brown and beige adipogenesis suggest that the control of adaptive thermogenesis is more complex than is currently appreciated. Cardiomyocytes, a cell type that is thought to have little crosstalk with adipocytes, can markedly alter the gene expression and function of adipose through the secretion of potent cardiometabolic hormones. Importantly, cold increases the concentrations of natriuretic peptides, suggesting that this browning system may have evolved, perhaps in epicardial fat, to safeguard cardiac function in animals during cold exposure.(97)

Cyclooxygenase-2 (COX2, the rate-limiting enzyme in prostaglandin synthesis) and WAT-derived prostaglandins (PGE2 and PGI2) appear to be crucially involved downstream of β-adrenergic stimulation in the induction of UCP1 expression in adipocytes in inguinal WAT, but not in classic interscapular brown adipocytes.(122,123) Overexpression of COX2 in WAT results in WAT browning, increased systemic energy expenditure, and protection against dietary obesity.(122) Importantly, evidence has been obtained that COX-dependent induction of UCP1 in WAT is important for diet-induced thermogenesis and energy balance in obesity-resistant mice.(123)

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, a major ingredient in hot pepper widely used as a spice in food) and nonpungent capsaicin analogs (capsinoids) have an anti-obesity action in rodents linked to SNS activation. Reported effects of these compounds include activation of BAT thermogenesis and whole body energy expenditure in both rodents and humans (124), inhibition of adipogenesis (125) and stimulation of lipid catabolism (126) in white adipocyte cell models, and induction of browning of WAT in high-fat diet-fed rats supplemented with capsaicin (127). The bioactivity of capsaicin has been related to enhancement of catecholamine secretion from the adrenal medulla through

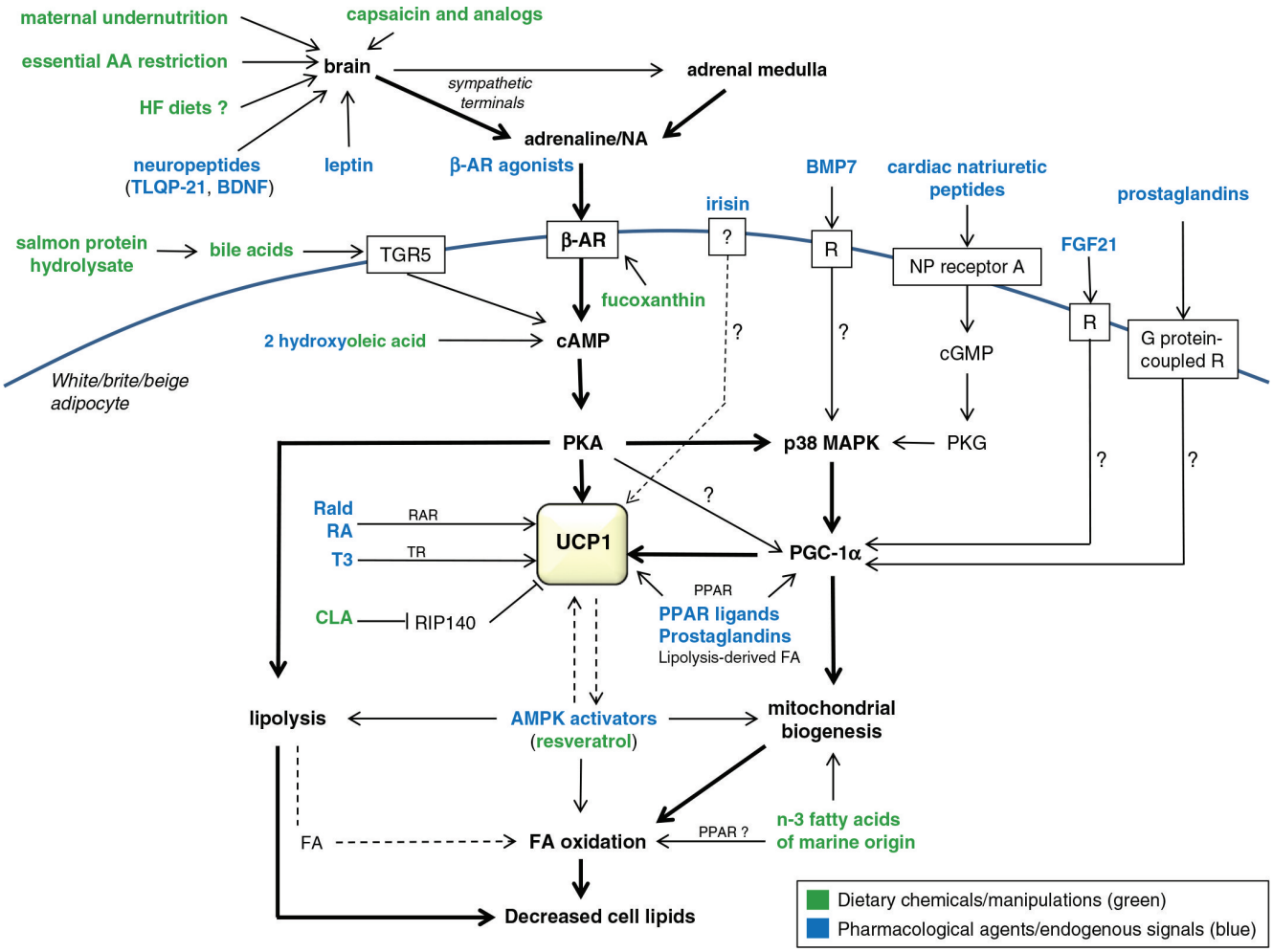

Figure 4. Pharmacological agents, endogenous signals and dietary chemicals/conditions that have been related to the browning of WAT.(121) (Adapted with permission from Elsevier)

activation of the SNS (128), and to binding to and activation of specific TRP channels within the gastrointestinal tract (47) and visceral adipose tissue (125).

Fucoxanthin is an allenic carotenoid present in the chloroplasts of edible brown algae. It is themost abundant of all carotenoids, accounting for more than 10% of the estimated total natural production of carotenoids.(129) Fucoxanthin metabolites accumulate in abdominal WAT. (130) Fucoxanthin or fucoxanthin-rich seaweed extract, alone or as part of mixtures with other selected agents, has been shown to counteract the development of dietary obesity and reduce abdominal WAT in obese animals through mechanisms that include the stimulation of WAT browning, with induction of UCP1 mRNA and protein expression and lipid catabolism-related genes in abdominal WAT.(131-135) Notably, fucoxanthin intake promotes WAT browning at doses at which it does not affect UCP1 expression in BAT, suggesting a WAT selective effect.(131)

Stimulatory effects of olive oil on the expression of UCPs have been described. Its previously demonstrated capacity to enhance the cyclic adenosine monophosphate (cAMP)/Protein Kinase A pathway (136) since phosphorylation of cAMP response element-binding protein (CREB) was drastically increased together with UCP1 expression in WAT of treated rats (137).

Conjugated linoleic acid is a group of dietary fatty acids that have received considerable attention due to their ability to significantly reduce adipose mass in a variety of species. The mechanisms by which CLA (specifically the trans-10, cis-12 isomer) depletes adipose mass may include a combination of increased apoptosis, decreased preadipocyte differentiation and lipogenesis, and increased fatty acid oxidation and energy expenditure in white adipocytes.(138) Intake of long-chain n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) (20:5 n-3) and docosahexaenoic acid (DHA) (22:6 n-3), which are abundant in marine fish oil, reduces obesity and visceral fat mass in rodents and, although evidence is limited, possibly also in humans.(139) The anti-adiposity effect of n-3 PUFA does not result from a reduction in food intake, but rather

reflects metabolic changes in several tissues (139), including increased UCP1-mediated adaptive thermogenesis in BAT (140) and oxidative metabolism in WAT (141).

Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring polyphenol present in grapes and other food vegetables, has many remarkable effects on energy metabolism and related aspects in mammals. These effects are believed to be due, at least in part, to resveratrol's ability to activate 5' AMP-activated protein kinase (AMPK) (possibly by interfering with mitochondrial respiration thus leading to an AMP/ATP imbalance) and, downstream of AMPK, sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1 - alpha (PGC-1α). (141) Resveratrol treatment has been shown to result in increased mitochondrial content and activity in skeletal muscle (142), liver (143) and BAT (142) in rodents. Resveratrol also impacts on white adipocyte biology, as it may inhibit adipogenesis of preadipose cells (144-146) and enhance fat mobilization in fully differentiated fat cells through stimulation of adipose triglyceride lipase (147) and negative modulation of PPARγ stability and transcriptional activity (144,148,149). A browning effect is suggested by findings that resveratrol enhances mitochondrial DNA content and fatty acid oxidation together with UCP1 expression in mouse embryo fibroblast (MEF)-derived adipocytes (149) and UCP1 expression in maturing 3T3-L1 preadipocytes (150).

Conclusion

Knowledge regarding WAT browning and BAT activation could be useful in strategies to help prevent and manage obesity and related co-morbidities, such as type 2 diabetes and hepatosteatosis. Important advances have been made in the understanding of signaling pathways and compounds influencing browning of WAT and a large amount of data are currently available. Many treatments/manipulations that induce browning of WAT also induce the activity of classical

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